REMARKS

This Amendment, filed in reply to the Office Action dated February 26, 2008, is believed to be fully responsive to each point of objection and rejection raised therein. Accordingly, favorable reconsideration on the merits is respectfully requested.

Claims 1-8 are all the claims pending in the application. Claims 1-8 are rejected. Claims 1-8 are amended herewith. The amendments to Claims 3-8 are solely to improve clarity. Support for the amendment to Claim 1 can be found throughout the specification as originally filed, such as, for example, page 6, paragraph 2 to page 7, paragraph 2, and inherently within Applicants' working Examples. Support for the amendment to Claim 2 can be found throughout the specification, and at, for example, page 12, lines 5-12, of the specification as originally filed. No new matter is added by way of this amendment. Entry and consideration of this amendment are respectfully requested.

Information Disclosure Statement (IDS)

Applicants thank the Examiner for returning signed and initialed copies of the PTO Forms SB/08 that accompanied the Information Disclosure Statements filed January 27, 2005, and May 7, 2007.

However, the Examiner scored through the references listed on the PTO Form SB/08 that accompanied the Information Disclosure Statement filed September 24, 2004, because copies of the references were allegedly not submitted therewith. Of the references not considered by the Examiner, Applicants note that the Buchholz *et al.* reference was cited by the Examiner in the PTO Form PTO-892 that accompanied the Office Action, and thus need not be resubmitted. Additionally, Applicants note that the disclosures of EP 1105517 and JP 2002-523058 are

identical to that of the International Publication, WO 00/11208. Accordingly, Applicants submit herewith a copy of WO 00/11208, which has not been considered by the Examiner. Consideration of this reference is respectfully requested.

Claim to Priority

Applicants thank the Examiner for acknowledging Applicants' claim to priority, and receipt of the priority document from the International Bureau, namely Japanese Application No. 2002-082821, filed March 25, 2002.

Objections to the Claims

On page 2 of the Office Action, Claims 1-8 are objected to because the claims are allegedly not drafted in compliance with U.S. standard practice. Specifically, the Examiner objects to recitation of "being characterized by" in Claim 1. Further, the Examiner objects to recitation of "characterized in that" as recited in the remaining claims, and suggests replacing this phrase with "wherein."

Solely to advance prosecution, and without acquiescing in the rejection, Applicants herewith amend the claims in accordance with the Examiner's suggestion. Applicants respectfully submit that the amendments to the claims overcome the objection.

Withdrawal of the objection is respectfully requested.

Claim 2 is Definite Under 35 U.S.C. § 112, Second Paragraph

On page 2 of the Office Action, Claim 2 is rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. The Examiner asserts that the term "freeze-shattered" is not a conventional term used in the art, and thus is indefinite.

While Applicants believe that one of ordinary skill in the art would fully understand the bounds of the term "freeze-shattering," in the interest of compact prosecution, Applicants herewith amend Claim 2 to recite a freezing step and a pulverizing step, and a step of adding the pulverized biological sample to the perchloric acid solution to produce a "suspension." It is submitted that the amendment overcomes the rejection.

Withdrawal of the rejection is respectfully requested.

Claims 1-8 are Patentable Under 35 U.S.C. § 103

1. On page 3 of the Office Action, Claims 1-6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Buchholz *et al.* (*Anal. Biochem.*, 2001) (hereinafter "Buchholtz") in view of Hosokawa *et al.* (*Anal. Biochem.*, 1986) (hereinafter "Hosokawa").

In making the rejection, the Examiner alleges that Buchholz discloses a method for the quantification of intracellular metabolites in *Escherichia coli* K12 using liquid chromatographic-electrospray ionization tandem mass spectrometric techniques, including assaying concentrations of Coenzyme A molecules (acetyl-CoA). The Examiner further alleges that the method of Buchholz comprises adding cGMP as an internal standard to *E. coli* cell pellets, adding perchloric acid, freezing cells at -80°C, followed by centrifuging and neutralizing the supernatant and performing HPLC-ESI-MS-MS analysis.

However, the Examiner acknowledges that Buchholz does not specifically disclose a step of solid-phase extraction of the sample, or using a structural analog of Coenzyme A as an internal standard.

In an attempt to rectify the deficiencies of Buchholz, the Examiner cites to Hosokawa, who allegedly disclose the determination of short-chain acyl-coenzyme A esters by high-performance liquid chromatography. The Examiner alleges that Hosokawa disclose a method comprising extraction of acyl-coenzyme A from freeze-clamped rat livers using perchloric acid, applying the extract to a Sep-Pak C18 cartridge (which the Examiner understands to be solid phase extraction using an octadecylsilyl group), and eluting the extract with ethanol/water containing ammonium acetate. The Examiner asserts that the eluate is subsequently separated by reverse phase HPLC using columns.

The Examiner contends that it would have been obvious for a person of ordinary skill in the art to apply the step of solid-phase extraction disclosed by Hosokawa to the method of Buchholz, and that one of ordinary skill in the art would have been motivated to combine the methods in this manner because it would allow for better separation of such analogous compounds as malonyl-CoA, succinyl-CoA, acetyl-CoA, acetoacetyl-CoA, and propionyl-CoA, as indicated by Hosokawa.

Applicants respectfully disagree, and traverse the rejection in view of the following remarks.

In order to establish a *prima facie* case of obviousness, the references must, in combination, teach each and every limitation of the currently claimed invention, *In re Royka*, 490 F.2d 981, 985 (C.C.P.A. 1974). Second, the Examiner must provide sufficient reasons why one of skill in the art would combine the references to arrive at the claimed invention. Finally,

there must be a reasonable expectation of success in combining the references. *In re Vaeck*, 947 F.2d 488, 493 (Fed. Cir. 1991).

First, Applicants respectfully submit that one of ordinary skill in the art would not possess *any* motivation to combine the cited references in the manner claimed by the Examiner. In making the rejection, the Examiner contends that one of ordinary skill in the art would be motivated to combine the teachings of Buchholz and Hosokawa because Hosokawa allegedly disclose that the solid-phase extraction step allows "a better separation of such analogous compounds as malonyl-CoA, succinyl-CoA, acetyl-CoA, acetoacetyl-CoA, and propionyl-CoA." However, Applicants submit that one of ordinary skill in the art would not reasonably combine these teachings in the manner claimed, particularly in view of the disclosures of Buchholz and Hosokawa.

Specifically, as disclosed on page 49 of Hosokawa, the solid-phase extraction step produced "concentrated and partially purified CoA compounds in acid-extracted samples prior to HPLC analysis." (Emphasis added.) Further, as a result of this purification step, Hosokawa admit that ATP and ADP are not bound to the column. Even if such an approach were to be effective when specifically measuring CoA molecules by HPLC, due to a reduction in contaminating species, one of ordinary skill in the art would have no motivation to combine the solid-phase extraction step of Hosokawa with the method of Buchholz because the method of Buchholz is directed towards "accurately [determining] the concentration of a large number of reactants (i.e., metabolites, nucleotides, cofactors) in order to understand "*in vivo*" reaction kinetics." (Emphasis added.) Buchholz *et al.*, Abstract. One of ordinary skill in the art would thus realize that the method of Hosokawa purifies CoA molecules from other reactants, and thus would destroy the operability of the method of Buchholz for the intended purpose. As a specific

example, on page 47 of Hosokawa, it is disclosed that "[w]e found that CoASH and its acyl thioesters in acidic water are bound to a Sep-Pak C_{18} cartridge equilibrated with acidic water, but ATP and ADP are not." However, in the method of Buchholz, as evidenced in Table 1, Figure 8, Table 3, Table 4, Figure 13, and throughout the text, the measurement of ADP and ATP is performed by Buchholz. As ATP and ADP are important measurements in understanding microbial metabolism, as disclosed by Buchholz, introduction of the solid-phase extraction step of Hosokawa would purify the sample to such an extent that several reactants essential to the experiments of Buchholz would be eliminated prior to HPLC-MS-MS analysis, which include at least ATP and ADP. In this regard, Applicants note that "[if a] proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification." *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed.Cir.1984). Thus, Applicants respectfully submit that one of ordinary skill in the art would not have possessed *any* motivation to combine the methods of Buchholz and Hosokawa, and hence the rejection is improper for at least this reason. Further, as a result of such lack of motivation, one of ordinary skill in the art would not have a reasonable expectation of success in combining the references, as is also required to maintain a finding of obviousness. Applicants respectfully submit that the claims are not obvious at least in view of the foregoing.

In addition, Applicants note that although Buchholz, on page 131, disclose that "cGMP [was added] as [an] internal standard," the quantification method of Buchholz does not use cGMP concentration to determine the concentrations of reactants, but rather, as disclosed on page 131, "[q]uantification was accomplished via the [M-H]- ion by applying the standard addition method. A standard solution containing **all analytes** at a known concentration was prepared. By spiking cell extracts with increasing amounts of this standard solution, linear

regression plots of peak area versus concentration were obtained." (Emphasis added.) Thus, in contrast to the instant invention, the method of Buchholz uses the same analytes as are present in the sample, and would therefore not be distinguishable from the analyte in the sample in the absence of multiple samples spiked with varying amounts of analyte.

In contrast to Buchholz, Applicants' invention employs a structurally distinct analyte as a standard (e.g. ^{13}C-labeled), avoiding the need for multiple samples having different concentrations of standard. Thus, to even further clarify Applicants' claimed invention, Claim 1 is amended herewith to recite "wherein said internal standard substance is not present in said biological sample prior to said extraction using a strongly acidic solution."

Applicants respectfully submit that the claims are not rendered obvious by the cited references at least in view of the foregoing.

Withdrawal of the rejection is respectfully requested.

2. On page 4 of the Office Action, Claims 7 and 8 are rejected under 35 U.SC. 103(a) as being unpatentable over Buchholz in view of Hosokawa, as applied to Claims 1-6 above, and further in view of Reszko *et al.* (*Anal. Biochem.*, 2001) (hereinafter "Reszko").

Buchholz and Hosokawa are relied upon for the same reasons as in the rejection of Claims 1-6. However, the Examiner acknowledges that neither Buchholz nor Hosokawa disclose substituting at least three carbons in the main carbon chain of a CoA analog with ^{13}C.

In an attempt to rectify the deficiencies of the primary references, the Examiner cites to Reszko, who allegedly disclose "assay of the concentration [of,] and ^{13}C-isotopic enrichment of[,] malonyl-coenzyme A by gas chromatography-mass spectrometry." The Examiner alleges that the method of Reszko comprises perchloric acid extraction of a tissue, spiking the tissue

extract with a [U-$^{13}C_3$]malonyl-CoA or dimethylmalonyl-CoA internal standard, and isolation of short-chain acyl-CoA fractions on an oligonucleotide purification cartridge.

In view of the above, the Examiner asserts that it would have been obvious for a person of ordinary skill in the art to introduce the step of ^{13}C-isotopic enrichment of coenzyme A ester analog into Buchholz-Hosokawa's method, "because it allows investigating the origin of the acetyl moiety of malonyl-CoA using mass spectrometry, as indicated by Reszko (page 69, right column)."

Further, the Examiner alleges that Reszko discloses that in lipogenic organs, a minimum value of the turnover of malonyl-CoA can be estimated from the rate of fatty acid synthesis measured by the incorporation of ^2H or ^3H from deuterium- or tritium-enriched water. From this, the Examiner asserts that a person of ordinary skill in the art would readily use a deuterium-enriched internal standard instead of a ^{13}C-enriched internal standard, because the former is useful for the analysis of malonyl-CoA from lipogenic organs, and is much easier to obtain than ^{13}C-enriched standards.

Applicants respectfully disagree, and traverse the rejection in view of the following remarks.

Initially, Applicants respectfully submit that Reszko does not overcome the lack of motivation to combine the primary references, nor does it restore operability to the method of Buchholz. Indeed, the method of Reszko further destroys the operability of Buchholz, because akin to Hosokawa, Reszko selectively purify short-chain acyl-CoA molecules with an oligonucleotide purification cartridge. Thus, Applicants respectfully submit that Claims 7 and 8 are not rendered obvious at least for this reason.

Further, Applicants note that the Examiner's rationale for combining Reszko with Buchholz and Hosokawa is that one of ordinary skill in the art would be motivated to introduce the "assay of the concentration and ^{13}C-isotopic enrichment of malonyl-coenzyme A by gas chromatography-mass spectrometry" as disclosed by Reszko, comprising perchloric acid extraction of a tissue, spiking the tissue extract with a [U-$^{13}C_3$]malonyl-CoA or dimethylmalonyl-CoA internal standard, and isolation of short-chain acyl-CoA fractions on an oligonucleotide purification cartridge, "because it allows investigating the origin of the acetyl moiety of malonyl-CoA using mass spectrometry, as indicated by Reszko (page 69, right column)."

Applicants respectfully disagree with the Examiner's position, and submit that one of ordinary skill in the art would have no motivation to combine the references in the manner suggested by the Examiner. Specifically, although Reszko admit that "the origin of the acetyl-CoA and malonyl-CoA in nonlipogenic organs is not known," one ordinarily skilled in the art would not perform the perchloric acid extraction, spiking and cartridge purification steps disclosed by Reszko to determine as such, as posited by the Examiner. Indeed, performing these steps would provide no information as to the origin of the acetyl moiety of malonyl-CoA because the mere spiking of samples with [U-$^{13}C_3$]malonyl-CoA or dimethylmalonyl-CoA does not in itself label any native malonyl-CoA in the sample to allow such a determination.

Such is confirmed in the portion of Reszko referred to by the Examiner (i.e., page 69, right column) in which Reszko disclose that "[the origin of the acetyl moiety of malonyl-CoA in nonlipogenic organs] could be investigated using assays for the ^{13}C-labeling and mass isotopomer distribution [of] malonyl-CoA. For example, the turnover of malonyl-CoA could be measured by pulse or continous labeling with $NaH^{13}CO_3$. This is why we developed [GC-MS]

assays for the concentration and mass isotopomer distribution of malonyl-CoA. We then demonstrated the usefulness of these assays by labeling malonyl-CoA from $NaH^{13}CO_3$, [3-^{13}C]lactate + [3-^{13}C]pyruvate, and [U-$^{13}C_2$]acetate in perfused rat livers and from $NaH^{13}CO_3$ in perfused rat hearts." Thus, one of ordinary skill in the art would readily understand that the origin of the acetyl moiety of malonyl-CoA is not achieved by the spiking of the sample with [U-$^{13}C_3$]malonyl-CoA or dimethylmalonyl-CoA, but rather, relies upon modification of the malonyl-CoA in living cells by perfusion of rat livers and hearts with isotopic malonyl-CoA precursors.

Accordingly, Applicants respectfully submit that the Examiner's rationale for combining Reszko with Buchholz and Hosokawa is technically unsound, and in fact illogical. Indeed, spiking of a tissue that has already been extracted with perchloric acid with [U-$^{13}C_3$]malonyl-CoA or dimethylmalonyl-CoA would provide no information as to the origin of the acetyl groups on the malonyl-CoA therein because (a) [U-$^{13}C_3$]malonyl-CoA or dimethylmalonyl-C would not transfer ^{13}C groups to unlabeled malonyl-CoA in the sample, and (b) even if [U-$^{13}C_3$]malonyl-CoA or dimethylmalonyl-C were suitable as donors for this purpose, which they are not, these compounds would need to be added to *living* cells, not to a perchloric acid-extracted sample. Accordingly, in view of the above, one of ordinary skill in the art would not possess *any* expectation of success in combining the method of Reszko with Buchholz and Hosokawa in the manner suggested by the Examiner, as is required to maintain such a rejection.

Further, with regard to the Examiner's assertion that one of ordinary skill in the art would be motivated to use a deuterium-enriched internal standard instead of a ^{13}C-labeled standard, Applicants respectfully submit that the Examiner's reasoning is again technically unsound. The portion of Reszko cited by the Examiner refers to the addition of deuteriated or tritiated water to lipogenic organs undergoing fatty acid synthesis. One of skill in the art would understand that

addition of a deuterium-enriched internal standard as claimed would be useless in the method disclosed by Reszko, since the method of Reszko relies upon incorporation of ^{2}H or ^{3}H from isotopic water molecules during fatty acid synthesis. One of ordinary skill in the art would readily understand that the claimed internal standards would not transfer ^{2}H or ^{3}H to fatty acids in the method suggested by the Examiner because (a) the claimed internal standards would not be suitable donors and (b) even if the internal standards were suitable as donors for this purpose, which they are not, these compounds would need to be added to living cells undergoing fatty acid synthesis, not to a perchloric acid-extracted sample. In view of the foregoing, Applicants respectfully submit that Claims 7 and 8 are not rendered obvious by the cited references.

Withdrawal of the rejection is respectfully requested.

Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

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